STUDY ON ISOLATION OF *Pyricularia oryzae* CAUSED BLAST DISEASE OF RICE AND ITS MANAGEMENT BY USING POTASSIUM SILICATE

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STUDY ON ISOLATION OF *Pyricularia oryzae* CAUSED BLAST DISEASE OF RICE AND ITS MANAGEMENT BY USING POTASSIUM SILICATE

ABSTRACT

Blast of rice caused by *Pyricularia oryzae* fungus which played vital roles in reducing yield of rice in Bangladesh from last two years. The prevalence of rice blast was remarkable in greater Dinajpur district including Thakurgaon and Rangpur. The blast samples were collected from the farmers field of selected area of Dinajpur, Thakurgaon and Rangpur districts which were reported to have blast infection by the regional agricultural extension centers during Boro season 2017-2018. Blast infected leaf, node and neck samples were collected for the *in-vitro* study. The *in-vitro* study was carried out at the Molecular biology and Plant virology laboratory, Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. The main objectives of this experiment was to isolate the blast pathogen and to identify the best culture media for pathogenic growth and sporulation of *Pyricularia oryzae*, to study its morphological and pathogenic characteristics. From the study, it was revealed that Carrot Rice straw Dextrose Agar (CRDA) was found for the best mycelial growth and sporulation of pathogen. The morphological study of the derived three isolates from the field samples, it was evident that the disease was caused by *Pyricularia oryzae*, as the isolates produced hyaline, pyriform three celled conidia (average 16.45 × 7.46 µm) from leaf sample and CRDA media, respectively. They were able to re-establish the disease in *in-vitro* pathogenicity test. *In-vivo* study was also carried out by using Blast susceptible rice variety BRRI dhan 28 to assess the efficacy of Potassium Silicate (K_2SiO_3) in different doses (0, 1, 2, 4, 8, or 10 g L^{-1} Si), number of sprayings at pH 5.5. Silicon (Si) is known as a “beneficial element” for plants. The direct and indirect benefits of the element for crops (especially grasses) are related to resistance to diseases, pests a drought. From this study it was found that spraying of K_2SiO_3 at 4 g L^{-1} significantly reduce the blast disease incidence and severity.

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ABBREVIATIONS AND ACRONYMS

% : per cent

^{0}C : Degree Celsius

μm : micrometer

cm : centimeter

g : gram

h : hours

ha : hectare

i.e., : that is

l : liter

μl : microliter

°C : degree celcius

mg : milligram

ml : milliliter

mm : millimeter

MT : Million Tonns

CRD : Completely Randomized Design

RBD : Randomized Block Design

LSD : Least Significant Difference

DS : Disease severity

DI : Disease incidence

DAT: Days After Transplanting

DAS: Days After Sowing

DAI: Days After Imergence

et al. : and others

No. : Number

RH : Relative Humidity

Viz., : Namely

BBS : Bangladesh Bureau of Statistics

BER : Bangladesh Economic Review

SRDI : Soil Resource Development institute

IRRI: International Rice Research Institute

BRRI: Bangladesh Rice Research Institute

FAO : Food and Agriculture Organization

INTRODUCTION

Rice (*Oryza sativa* L.) is the source of subsistence for more than one third of human population. It is the main staple food in the Asia and the Pacific region, providing almost 39 % of calories (Yaduraju, 2013). Two types of rice is cultivated world wide Indica and Japonica. These plants are native to Tropical and Subtropical Southern Asia and Southeastern Africa, respectively (Linares, 2002). More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live (Kole, 2006).

Bangladesh is an agro-based developing country and still striving hard for rapid development of its economy. Agriculture is the mainstay of Bangladesh economy and it contributes about 22 percent of the gross domestic product (GDP). Approximately, 12 percent of GOP has been derived from crops while rice alone contributes 9.5 percent to the agricultural GDP (BBS, 2016). Almost all of the 13 million farm families of the country grow rice. About 75% of the total cropped area and over 80% of the total irrigated area is planted to rice (BBS, 2016). Globally rice occupies an area of 163 m ha with a production of 719 MT of paddy (FAO, 2016). Asia is the leader in rice production accounting for about 90 per cent of the world's production. Over 75 per cent of the world supply is consumed by people in Asian countries and thus rice is of immense importance to food security of Asia. In Asian countries more than 80% people taken rice in their daily diet as a staple food. The global annual demand for rice is expected to be around 800 million tons by 2025 in view the expected increase in population. Rice is a major staple food and a mainstay for the poor rural population and their food security. Developing countries account for 95% of the total production, with China and India alone responsible for nearly half of the world output. It is widely cultivated in India, China, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Japan, Philippines and Brazil. China is the leading rice producer followed by India, Indonesia and Bangladesh in 2016 (FAO, 2017).

Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%. Rice grain contains on an average 7% protein, 62-65 % starch, 1.3% fibre and0.7% fat. It is also a main source of vitamin B1 (thiamin), B2 (riboflavin), B3 (niacin) and B5 (pantothenic acid).The Biological value of rice is 63% whereas Biological value of wheat and maize is 49% & 36% respectively. In Bangladesh more than 95% of population consumes rice and it alone provides 76% of calorie and 66% of total protein requirement of daily food intake (Bhuiyan *et al*., 2002). Thus, rice plays a vital role in the livelihood of the people of Bangladesh.

Rice is grown on about 10.5 million hectares which has remained almost stable over the past three decades. The country is now producing about 25.0 million tons to feed her 160 million people (Hossain *et al.,* 2017). This indicates that the growth of rice production was much faster than the growth of population. This increased rice production has been possible largely due to the adoption of modern rice varieties on around 66% of the rice land which contributes to about 73% of the country's total rice production (BBS, 2016). Rice, however, continues to remain main sources of food supply for the people of all the sectors, producing nearly 95 percent of the total food requirements, but there is still a need to increase production to feed the growing population which increases at the rate of 1.32 percent per annum (BER, 2010).The International Rice Research Institute (IRRI), Philippines, estimates that in order to feed the growing global population, rice production must increase by another one-third by the year 2020. Rice is known to be attacked by many pests and diseases which cause huge losses annually worldwide. Rice crop is subjected to attack by 50 diseases include 21 fungal,6 bacterial, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Jabeen *et al*., 2012). Among the major rice diseases that often cause great economic losses, rice blast (*Pyricularia oryzae*) is a vicious threat to the country's

economy (Ganesh *et al.,* 2012). Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world.

It is estimated that each of the three years enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler *et al.*, 1994). Out of the total yield loss due to diseases in rice, 35% is by blast, 25% by sheath blight, 20% by BLB, 10% by tungro and remaining 10% by other diseases. The disease causes yield losses from between 1-100% in Japan (Kato, 2001), 70% in China, 21-37% in Bali Indonesia and 30-50% in South America and Southeast Asia (Baker *et al.*, 1997).

During 2016, serious yield losses occurred due to epiphytotic of blast diseases caused by *Pyricularia oryzae* have been recorded in different regions in Bangladesh such as Dinajpur, Rangpur, Thakurgaon, Panchghar, Kushtia, Jashore, Pabna, Borishal, Mymensingh, Munshigonj ,Chuadanga etc. and the production of Boro rice and transplanted Aman in Bangladesh were greatly reduced due to blast infestation with 21.19% and 11.98% disease severity. Most popularly affected Boro rice was BRRI dhan28 (29.6% disease severity) followed by BRRI dhan 29 (25.9% disease severity) BRRI dhan61 (21.9% disease severity) and T. Aman rice was BRRI dhan34 (22.9% disease severity) (Hossain and Ali, 2017). The major symptoms of this disease are found on leaves with brownish spot having grey center, sunken lesion on node and brown or black lesion found on neck of the panicle. Among them panicle blast is most devastating and sometimes causes 100 % yield loss. The occurrence of this disease increased the market value of rice which was a threat for the national food security and economy.

By considering the circumstances, the prime aim of the study was to find out the suitable culture media for growing and sporulation of blast pathogen in *in-vitro* conditions. The other major target of this study was to minimize the yield losses through development of induce resistant system in rice plant by using potassium silicate as foliar spray.

OBJECTIVES

Keeping these facts in view the present investigation was carried out with the following objectives:

-To find out a suitable culture medium for the mycelial growth and sporulation of blast pathogen, *Pyricularia oryzae.*

-To study the cultural and morphological variability of this pathogen.

-To assay the efficacy of potassium silicate to manage blast disease in *in-vivo.*

REVIEW OF LITERATURE

The available literature of work done on blast disease of rice and its management strategies have been reviewed in this chapter. The review of literature pertaining to this dissertation is presented in the following headings and sub-headings.

2.1. Importance of rice

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world and is consumed by 50% of the world population (Luo *et al*., 1998). There are two species cultivated *Oryza sativa* L (Asian rice) and *Oryza glaberrima* S (African rice) (Silue and Notteghem, 1991).

Oryza glaberrima is traditionally found in diverse West African agro ecosystems but it is largely abandoned in favor of high yielding Oryza sativa cultivar that has higher agronomic performance (Seebold *et al*., 2004). However, *Oryza sativa* cultivars are often not sufficiently adapted to various abiotic and biotic conditions in Africa. *Oryza glaberrima* has been found to have several useful traits like being moderate to high in their level of resistance to blast (Silue and Notteghem, 1991), rice yellow mottle virus (Attere and Fatokun, 1983); (Jabeen*et al.,* 2012), rice gall midges, insects (Alam, 1988) and nematodes (Ram *et al.*,2012). The variety has also been found to be tolerant to abiotic stresses such as acidity, iron toxicity, drought, and weed competition (Sano *et al.* 1984; Jamal-u-Ddin *et al.*2012).

Rice is the most economically important staple food crop in Bangladesh, India, China, East-Asia, South East Asia, Africa and Latin America catering to nutritional needs of 70% of the population in these countries (FAO,2009). It is the main staple food in the Asia and the Pacific region, providing almost 39 % of calories (Yaduraju, 2013).

In several developed countries such as North America and European Union (EU) also, rice consumption has increased due to food diversification and immigration (Dubey S.C., 2005).

Worldwide, rice is grown on 161 million hectares, with an annual production of about 678.7 million tons of paddy (FAO, 2009). About 90% of the world's rice is grown and produced (143 million ha of area with a production of 612 million tons of paddy) in Asia (FAO, 2009). Five Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%. During 2012-13 and 2013-14, the world production has increased by 1% (from 472 Million Tonnes to 476 Million Tonnes), trade by 8% (from 38 Million Tonnes to 41 Million Tonnes) and consumption by 3% (from 469 Million Tonnes to 481 Million Tonnes) (Commodity profile for rice - January 2015). Rice is one of the three most important food crops of the world and the main staple food for nearly a half of the world's population (Von Braun, 2007).

2.2. Importance of blast disease of rice

Magnaporthe grisea (Anamorph *Pyricularia grisea* Sacc. synonym *Pyricularia oryzae* Cav.) causes rice blast disease in rice cultivation areas worldwide (BBS, 2017; Chiba *et al*., 1996; Kato, 2001). The disease causes yield losses from between 1- 100% in Japan (Kato, 2001), 70% in China, 21-37% in Bali Indonesia, 30-100% in Bangladesh (Singh G. and Prasad C.S. 2015) and 30-50% in South America and Southeast Asia (Baker *et al*., 1997).

Rice blast is one of the most important diseases of rice, caused by the fungus *Pyricularia oryzae* (Couch and Kohn 2002). One of the main limitations in production is rice blast disease caused by the fungus *Pyricularia oryzae*. Annual rice losses caused by this fungus during 90's had been estimated at 35% of the worldwide production (Oerke and Dehne 2004). In West Africa, the largest area of African production, this pathogen is the main constraint to production with yield losses ranging from 4-77%. The fungus is able to infect plants at all stages of growth and development in both upland and lowland rice production systems. Lowland rice produced in temperate and subtropical climates of Asia are highly susceptible to the pathogen, while tropical upland areas are susceptible only under irrigation (Nutsugah *et al*., 2008).

Blast disease caused by *Pyricularia oryzae* Cavara [Synonym *Pyricularia grisea* Sacc.,] the anamorph of *Magnaporthe grisea*, upsets production statistics of rice in Pakistan (Tirmali and Patil ,2000). In Pakistan during the last two decades, rice blast is 6 mostly found in districts of Faisalabad, Toba Tek Singh, Vehari and place like Gaggoo Mandi (Ali *et al.,* 2009).

The fungus *Pyricularia oryzae* attacks at all stages of the crop and symptoms appear on leaves and nodes (Seebold *et al.,* 2004). The symptoms are more severe in case of neck blast that is characterized by the infection at the panicle base and it's rotting (Bonman *et al.,* 1989). Heavy yield losses have been reported in many rice growing countries. For example 95, 50 and 40 percent grain loss may occur in Bangladesh and India (Padmanabhan, 1965), Philippines (Ou, 1985) and Nigeria (Arshad, 2008).

The most usual approaches for the management of rice blast disease include planting of resistant cultivars, application of fungicides and manipulation of planting times, fertilizers and irrigations (Georgopoulos and Ziogas, 1992; Mbodi *et al*., 1987; Naidu and Reddy, 1989).

Blast is known to attack nearly all above ground parts as well as during all growth stages of plant. Recent reports have shown that the fungus has the capacity to infect plant roots also (Shafaullah *et al*., 2011). The infection of rice blast occur when fungal spores land and attach themselves to leaves using a special adhesive released from the tip of each spore (Hamer *et al*, 1988).

The germinating spore develops an appressorium, a specialized infection cell which generates enormous turgor pressure (up to 8MPa) that ruptures the leaf cuticle, allowing invasion of the underlying leaf tissue (Dean, 1997; Hamer *et al.*, 1988).

2.3. Nature and disease symptoms

The pathogen may infect all the above ground parts of a rice plant at different growth stages: leaf, collar, node, internode, base, or neck, and other parts of the panicle, and sometimes the leaf sheath (Peterson L.G., 1994). The symptoms are

more severe in case of neck blast that is characterized by the infection at the panicle base and it's rotting (Bonman *et al.*, 1989).

Pyricularia oryzae infects and produces lesions on the following parts of the rice plant: leaf (leaf blast), leaf collar (collar blast), culm (culm nodes), panicle neck node (neck rot) and panicle (panicle blast). In leaf blast initial lesions/spots are white to gray-green with darker borders. Older lesions are white-grey, surrounded with a red-brown margin and are diamond shaped (wide centre and pointed toward either end). Lesion size is commonly 1-1.5 cm long and 0.3-0.5 cm wide. Under favorable conditions, lesions can coalesce and kill the entire leaf. In case of collar rot, lesions are located at the junction of the leaf blade and leaf sheath and can kill the entire leaf (Padmanabhan, 1974).

Infection to the neck node produces triangular purplish lesions, followed by lesion elongation to both sides of the neck node, symptoms which are very serious for grain development. When young neck nodes are invaded, the panicles become white in color the so called 'white head' that is sometimes misinterpreted as insect damage. Infected panicles appear white and are partly or completely unfilled. The whitehead symptoms can easily be confused with a stem borer attack which also results in a white and dead panicle. Panicle branches and glumes may also be infected. Spikelets attacked by the fungus change to white in color from the top and produce many conidia, which become the inoculum source after heading. Panicle blast symptoms include the panicle appearing 11 brown or black. Node infection includes infected nodes appearing black-brown and dry and often occur in a banded pattern. This kind of infection often causes the culm to break, resulting in the death of the rice plant. The pathogen is most common on leaves, causing leaf blast during the vegetative stage of growth, or on neck nodes and panicle branches during the reproductive stage, causing neck blast (Bonman, 1992).

Leaf blast lesions reduce the net photosynthetic rate of individual leaves to an extent far beyond the visible diseased leaf fraction (Bastiaans, 1991). Neck blast is considered the most destructive phase of the disease and can occur without being

preceded by severe leaf blast (Zhu *et al*., 2005). The neck blast infects the panicle causing failure of the seeds to fill or causing the entire panicle to fall over as it is rotted. Infection of the necks can be very destructive and directly reduces the economic value of the produce. The lesions are often greyish brown discoloration of the branches of the panicle and over time, the branches may break at the lesion. Out of three symptoms, neck blast is more destructive (Zhu *et al*., 2005).

The most important fungal pathogen *Pyricularia oryzae* of rice because of its widespread occurrence and destructive nature (Manibhushan Rao K.,1994). The fungus can attack any aerial part of the rice plant, including seeds. They also suggested systemic transmission of the fungus from seeds to seedlings.

The fungus *Pyricularia oryzae* was able to infect and produce lesions on all organs of the rice plant and when the fungus attacks young leaves, purple spots could be observed changing into spindle shape which has a grey center and purple to brown border. Brown spots appeared only on older leaves or leaves of resistant cultivars. In young or susceptible leaves, lesions coalesce and cause 8 withering of the leaves, especially at seedling and tillering stage. Infection to the neck results formation of triangular purplish lesions followed by elongation on both sides of neck. When young necks are infected, the panicles become white in color and later infection caused incomplete grain filling and poor grain quality (Hajimo, 2001).

Leaf blast fungus can attack the rice plant at any growth stage and can cause severe leaf necrosis and impede grain filling, resulting in decreased grain number and weight. When the last node is attacked, it causes partial to complete sterility. Rice blast pathogen infect all the above ground parts of rice plants at different growth stages, i.e., leaf, collar, nodes, internodes, base or neck and other parts like panicle and leaf sheath. It starts a typical blast lesion on rice leaf as grey at the center with a dark border and is spindle shaped (Ram *et al.*,2007). The environment with frequent and prolonged dew periods and with cool temperature in day time is most favorable for the spread of the disease (Castilla *et al.*, 2009).

2.4. Occurrence and distribution

Rice blast disease is distributed in about 85 countries in all continents where the rice plant is cultivated, in both low land and upland conditions. Rice blast is present wherever rice is cultivated, but the disease occurs with highly variable intensities depending on climate and cropping system. Environments with frequent and prolonged dew periods and with cool temperature in daytime are more favorable to blast (Chiba *et al.,* 1996; Liu *et al*., 2004).

In Pakistan during the last two decades, rice blast is mostly found in districts of Faisalabad, Vehari and places like Gaggoomandi (Arshad *et al*., 2008). Rice blast has been recorded in the Northern Territory (Stahl 1955; Heaton 1964), Brazil (Prabhu and Morais,1986), Queensland, Australia (Perrot and Chakraborty 1999; You *et al*., 2012), Sri Lanka (Senadhira *et al*., 1980), Colombia, Philippines, Japan, South Korea (Ou, 1985; Pena *et al*., 2007), Egypt (Reddy and Bonman, 1987; Sotodate *et al.*, 1991), China (Ling K.C., 2011).

Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world. Rice blast is a widespread and damaging disease of cultivated rice caused by the fungus *Pyricularia oryzae* (Ravindramalviya, 2014). It is the most destructive pathogen of rice worldwide. Around 50% of production may be lost in a field moderately affected by infection (Supriya Devi and Sharma, 2010).

It is estimated that each year enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler *et al*., 1994).

It was first reported as rice fever disease in China by Soon ying-shin in 1637 (Ou, 1985), in Japan it was reported as Imochi-byo by Tsuchiya in 1704. In India it was first reported in Thanjavur delta of Tamil Nadu in 1913 (Padmanabhan, 1965). It is a disease of immense importance in temperate, tropical, subtropical Asia, Latin America and Africa and found in approximately 85 countries throughout the world (Kapoor Pooja and Abhishek Katoch, 2014).

The disease is also a major problem in Penna river belts and Godavari in Andhra Pradesh. The blast fungus can attack more than fifty other species of grasses. It causes disease at seedling and adult plant stages on the leaves, nodes and panicles. It appears in irrigated low land or rainfed upland rice as well as in submerged or deep water rice. Rice blast is the most serious disease found in the extensive rice areas of Latin America, Africa, and Southeast Asia and is a worldwide problem in rice production. Rice blast disease is a significant constraint to global food security and agricultural trade (Padmanabhan, 1965).

In the West African sub-region, blast is recognized as a primary constraint to rice production, causing 3.2-77.0 % yield losses (Tripathi SK and Jain AK. 2005). Various reports by Twumasi (1996, 1998), Twumasi and AduTutu (1995) and Nutsugah and Twumasi (2001) have identified the blast disease of rice as a serious threat to rice production in Ghana.

2.5. About *Pyricularia oryzae*

The fungus *Pyricularia oryzae* (Hebert, 1971) Barr (Anamorph: *Pyricularia grisea* (Cooke) Sacc is the causal agent of rice blast disease. The perfect stage of Pyricularia grisea was earlier named as *Ceratosphaeria grisea* (Habert, 1971). Later Yaegashi and Nishihara (1976) suggested the genus *Magnaporthe*.

Finally *M. grisea* proposed as a perfect stage of *Pyricularia grisea* (cooke.) Sacc instead of *Ceratosphaeria grisea*. The mycelium consists of septate, uninucleate, branched hyphae. However, as the fungus gets older, the hypha become brown. Generally, growth of the pathogen is relatively more on upper surface making the spot more dark on upper side. Conidiophores are simple, septate, basal portion being relatively darker. Conidia are pyriform in shape and hyaline in colour, produced acrogenously, one after another. Conidia is three celled, the middle cell being much wider and darker, and end cell germinates giving out germ tube. Conidia is rarely two celled or four celled.

Formation of intercalary or terminal chlamydospores is common, which are globose, thick walled and olive brown (You *et al.*, 2012).

Commonwealth mycological institute CMI (You *et al.*, 2012) description of the culture: Cultures greyish in color, conidiophores single or in fascicles, simple or rarely branched, show sympodial growth. Conidia formed singly at the tip of the conidiophore at points arising sympodially and in succession, pyriform to obclavate, narrowed toward tip, rounded at the base, three celled rarely one or two celled, hyaline to pale olive, 19-23 × 7-9 μm, with a distinct protruding basal hilum. Chlamydospores often produced in culture, thick-walled, 5-12 μm diameter.

Fungus produce sexual fruiting bodies called perithecia within 21 days. Perithecia are flask-shaped that carry asci containing ascospores, the products of meiosis. Ascospores are arranged as unordered octads or as larger populations of randomly selected ascospores (Talbot, 2003).

2.6. Isolation of *Pyricularia oryzae*

Padmanabhan *et al*. (1970) isolated *Pyricularia oryzae* from samples of diseased leaves, necks, and nodes of the infected rice plant on oat meal agar (OMA) with traces of biotin and thiamine (B and T). Cultures were purified by dilution method, and single spore isolates were grown and multiplied on OMA + B & T at 25^0C.

The panicles were collected with the symptoms of neck blast, washed once with sterile distilled water, and placed on moist filter paper in Petri dishes at room temperature to induce sporulation. Conidia from the lesion surface were spread onto 3% water agar with a sterile loop and incubated overnight. Single germinating conidium was isolated and transferred to potato dextrose agar (Xia *et al*.,1993).

Rice leaves infected with blast were collected by Bonman *et al.*, (1987) and isolated by placing each lesion in a moist Petri dish and incubated at 250C until sporulation. Conidia from the lesion surface were spread on to water agar and the germinating conidium was isolated and transferred to agar slants.

Leaves were collected and panicles infected with rice blast from rice cultivars obtained from germ plasm bank at the Centro Internacional de Agricultura Tropical (CIAT) and the International Rice Research Institute (IRRI) by Correa *et al.,* (1993). They derived cultures from either mass or single conidial isolates obtained from single lesions. Cultures were maintained on V8 juice agar and multiplied for inoculations on rice-polish agar at 28^0C under continuous light. They stated that *Pyricularia oryzae* expressed its virulence spectrum irrespective of geographical location.

Eight samples of rice leaves infected with blast were collected from commercial fields of upland rice cultivars in the state of Goias, Brazil (Silue and Notteghem, 2009). Monoconidial isolates were obtained by directly transferring one conidium per lesion on 5% water agar from two to three lesions per leaf. The isolates from panicles in the majority of the cases were obtained from one conidium per panicle. The collected isolates were conserved on sterilized filter paper discs in a freezer at -20 ± 10^0 C.

Blast affected leaves of rice cultivars were collected from rice fields in Guilan province of Iran. Leaf pieces with lesions were surface sterilized with 0.5% sodium hypochlorite solution, washed with sterile distilled water and placed on potato dextrose agar in Petri dishes at 25ºC for 2–3 days. Later, Petri dishes were incubated at 25ºC in the dark or artificial fluorescent light on a12 h light/dark photoperiod for 15–25 days. Monoconidial isolates of the recovered fungi were maintained on half-strength potato dextrose agar slants in test tubes as stock cultures (Motlagh and Javadzadeh, 2010).

Vanaraj *et al.* (2013) Blast lesions were surface sterilized with 0.1% mercuric chloride for 1 minute and placed over clean glass slides kept in sterile Petri dishes padded with moist cotton. The Petri dishes were incubated for 48 hours at room temperature (28±2°C). Single conidia were identified from the sporulating lesions using a stereomicroscope and aseptically transferred to potato dextrose agar (PDA) slants for maintenance.

The causal organism was identified as *Pyricularia oryzae* based on the spore morphology.

2.7. Sporulation of the pathogen

Culturing of different isolates of *Pyricularia oryzae* was studied by Vanaraj *et al*., (2013) and reported that colonies of *P. oryzae* appeared as white on oat meal, rice polish and malt extract agar, pinkish white on potato dextrose agar and whitish grey on rice agar. Spore induction was hastened on maize stem pieces than on rice and *Panicum repens.* When spores of 11 isolates of *P. oryzae* were compared, conidia of the isolate from *Pennisetum purpureum* were significantly bigger than the other isolates. The spores of rice isolates from Erode and *Gopichetti palayam* were significantly smaller in length and width.

Blast fungal isolates produced ring like, circular, irregular colonies with rough and smooth margins on oat meal agar media having buff color, greyish black to black color (Srivastava *et al*., 2014). The colony diameters of different groups ranged from 67.40 to 82.50 mm and the conidial shape of the different groups was pyriform (pear-shaped) with rounded base and narrowed towards the tip which is pointed or blunt. On oat meal agar, colony color of all the isolates was usually grey with good growth. All the isolates showed raised mycelial growth with smooth colony margin (Gashaw *et al*., 2014).

Colony color of all the rice blast (*P. grisea*) isolates was usually buff with good growth on Oat meal agar, greyish black with medium growth on host seed extract + 2% sucrose agar, the raised mycelial growth with smooth colony margin on potato dextrose agar and raised mycelium with concentric ring pattern on Richard's agar medium. On host seed extract + 2% sucrose agar all the blast pathogenic isolates showed black to greyish black color with smooth colony margin and good growth (Meena, 2005).

Mycelium in cultures was first hyaline in color, then changed to olivaceous, 1 – 5.2 μm in width, septate and branched. The spore measurements were 15 – 22 μm × 4 – 7 μm (Average, 17.4 μm × 5.2 μm) (Hossain, 2000).

Ram et al., (2012) found isolates of the fungus from different hosts differed in their response in media for mycelial growth and sporulation. Radial mycelial growth and days of sporulation of *P. grisea* were studied by culturing three fungal isolates from rice, finger millet and Panicum sp. on six different media: prune agar (PA), oat meal agar (OMA), potato dextrose agar (PDA), finger millet leaf decoction agar, finger millet polish agar (FPA) and finger millet meal agar. The highest RMG was found in the isolates from finger millet and the lowest in the isolates from rice. The shortest days of sporulation (1 week) was found in the isolate from rice and the longest (>2 weeks) in the isolate from finger millet. Among the different media used, PA and OMA were found to be the best for mycelial growth and sporulation of the isolates both from rice and finger millet. The shape, color and compactness of the fungal colonies varied with the media and isolates used. Cross inoculation studies showed that the fungus isolates from rice were able to infect all the plant species while isolates from finger millet were only able to infect three plant species (*E. coracana, Setaria sp. and E. indica)*.

2.8. Morphological characters of the pathogen

The morphology of *P. oryzae* spores was described which measured 16 – 33 × 5 – 9 μm. Usually 22 – 27 × 7 – 8 μm with a small basal appendage, other dimensions were, basal appendage 1.2 – 1.8 (1.6) μm in width, basal cell 4.8 – 11.5 (7.8μm), middle cell 1.8 – 11.5 (6.6 μm), apical cell 6 – 14 (7 μm) in length (Nishikado ,1917).

Aoki, (1955) measured 16 isolates in potato dextrose agar culture and showed that, the average length of the isolate ranged from 21.2 to 28.4μm, and the average width from 7.3 to 14 9.0μm.

Ono and Nakazato (1958) observed that, the size of conidia of *P. grisea* varied

with the culture media also. Hossain (2000) observed mycelium in cultures was first hyaline in color, then changed to olivaceous, 1 – 5.2 μm in width, septate and branched. The spore measurements were 15 – 22 μm × 4 – 7 μm (Average, 17.4 μm × 5.2μm).

Linear growth of the colonies of the *Pyricularia* isolated from rice was measured on standard medium agar, oat meal agar, french bean agar and decoction agar made out of the leaf material of rice. He also determined the weight of mycelial mat produced by the isolates in the standard medium, Richards's medium, Browns medium and decoctions of leaf material of rice. The isolates produced good growth on the decoctions of their host material (Reddy A. P. K. and Bonman J.M. 1987).

2.9. Cultural characterization of the pathogen

2.9.1. Growth of the pathogen in different media

Four culture media for the study of mycelial growth of *P. grisea* under in vitro was used (Ravindramalviya, 2014). Among them PDA media supported maximum mycelial growth followed by Richard's Agar medium after 168 hrs of incubation. Then sporulation of *P. grisea* was observed in traces in Potato dextrose agar medium and Richard's Agar medium after 168 hrs of incubation. However, Czapek-Dox medium was not found effective for both vegetative growth and sporulation of the test pathogen.

Mahdieh, (2013) reported that PDA culture medium could provide the best medium for *P. oryzae* vegetative growth, regardless of light condition. However, *P. oryzae* could sporulate when light was provided either continuously or at intervals. A combination of 16/8 hr light/darkness intervals and adding rice materials to culture media could induce *P. oryzae* for a better sporulation.

Arunkumar and Singh, (1995) studied *Pyricularia grisea (M. grisea*) from rice on different solid culture media. They found that, maximum colony diameter of rice isolate occurred on malt extract agar and Leonin agar.

Hossain, (2000) observed that among the non-synthetic media, potato dextrose

agar supported maximum radial growth (85.00 mm), next was host extract + 2 per cent sucrose agar medium (80.33 mm) followed by oat meal agar (75.00 mm).

Cruz *et al.,* (2009) observed the higher sporulation on wheat meal culture medium in alternate light, dark regime.

Culturing of different isolates of *Pyricularia oryzae* was studied by Vanaraj *et al.* (2013) and reported that colonies of *P. oryzae* appeared as white on oat meal, rice polish and malt extract agar, grey on potato dextrose agar and whitish grey on rice agar.

Xinfa *et al*. (1995) stated that *Pyricularia* isolates from hosts including rice and common weeds in rice fields sporulated abundantly on sterilized barley or sorghum grains.

2.10. Management of rice blast disease under pot condition

Chemical management is more effective for management of blast disease of rice caused by *Pyricularia oryzae* (Peterson, 1990; Saifulla and Seshadri, 1992; Sood and Kapoor, 1997; Vijaya, 2002; Tripathi and Jain, 2005; Swamy *et al.*, 2009). Varier *et al*., (1993) reported that seed treatment with Tricyclazole at 4 g/kg seed which proved effective after 40 days of sowing.

Pal, (2014) studied the effect of Potassium silicate to control the leaf blast of rice. He found it was highly effective.

Ravindramalviya (2014) tested newly evolved fungicides viz; Trifloxystrobin 25% + Tebuconazole 50% (Nativo 75 WG), Kresoxim methyl (Ergon 44.3 SC), Thifluzamide 24 SC, Metaminostrobin 20 SC, Azoxystrobin 25 SC (Amistar), Tricyclazole 75 WP (Beam), Carbendazim 50WP (Bavistin), Propiconiazole 25EC (Tilt) against leaf blast of rice under natural conditions. Among them Azoxystrobin and Tricyclazole shows better result.

Potassium silicate as most effective fungicide for the control of rice blast and increasing the yield (Singh and Prasad, 2010).

Rabicide 30WP, Nativo SC and Score 250 EC treatments were made with dose

rates of 3 g/liter of water, 0.8 gm/liter of water and 1.25 ml/liter of water and proved effective in all the three weeks in reducing the disease (Prabhu *et al*. 2003). Potassium silicate was found significantly superior in controlling the disease and also resulted in significant increase in yield in Potassium silicate sprayed plots (4 g L^{-1}) (Ganesh et al., 2012).

Sing *et al*., (2014) reported that application of Potassium silicate (1.01 ml l-1) and tricyclazole (0.6 g l-1) significantly reduced the blast severity by 19.5 and 20.06% as compared to 66.6% in untreated control.

Debashis Dutta *et al*., (2012) tested various fungicides viz., Nativo 75WG, Gain 75 WP, Score 250 EC, Hexacon Super 5% SC, and Tilt 25 EC against rice blast on MTU 7029 rice variety and applied fungicides with dose rates of 0.4 g l-1 , 0.6 g l-1 , 1.25 ml l-1 , 1.5 ml l-1 , 1 ml l-1 water respectively. All the fungicides proved to be effective in the management of rice blast disease but, Nativo, Gain and Score proved effective in all the three weeks in reducing the disease more in 3rd week with 10.15%, 12.85% and 11.46%.

A research was conducted to minimize the losses caused by *pyricularia oryzae* Potassium silicate was used at different doses (0, 1, 2, 4, 8, or 16 g L^{-1} Si), number of sprayings at two solution pHs. Rice (*Oryza sativa*), cultivar 'Metica-1' (susceptible to blast), was grown in pots in a completely randomized experimental design. Silicate was applied beginning at the 22nd day after emergence (DAE). The pathogen was inoculated on the 25th DAE. Disease incidence was evaluated ten days after inoculation. The greatest reduction on blast incidence was observed at 4 g Si L^{-1}, regardless of solution pH (Yang *et al*., 2011).

MATERIALS AND METHODS

The present study entitled "Study on isolation of *Pyricularia oryzae* caused blast disease of Rice and its management by using Potassium silicate" was carried out during 2017-18 at Sher-e-Bangla Agricultural University, Dhaka. The pot experiment was conducted at net house and the lab experiment was done in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The details of the materials used and methodology adopted during the study are described below with some headings and sub-headings.

3.1. Experimental Site and Duration

The experiment was conducted in two steps. *In-vitro* experiment was conducted in Plant Pathology Laboratory and *In-vivo* experiment was conducted at net house. The experimental field area was situated at 23°46' N latitude and 90°22'E longitude at an altitude of 8.6 meter above the sea level (Anon. 2013). (Appendix-I).The experiment was conducted during the period of April 2017 to May 2018.

3.2. Soil Characteristics

The soil of the experiment was taken from a medium high land which belongs to the Modhupur tract, Agro Ecological Zone no 28. The soil texture was silt loam, High level of nutrients, non-calcareous, acidic, brown or red soil of Tejgaon soil series with a pH 6.7. Before conducting the experiment Soil samples of the experimental pots were collected from the experimental field of Sher-e-Bangla Agricultural University (SAU) at a depth of a 0 to 30 cm and analyzed in the Soil Resources Development Institute (SRDI), Farmgate, Dhaka. (Appendices- II).

3.3. Climate

The climate of the Madhupur Tract varies slightly from north to south, the northern part reaches being much cooler in winter. Average temperatures vary from 28^0 C to 32^0 C in summer, reducing to 20^0 C in winter, with extreme lows of 10^0 C. Rainfall ranges between 1,000 mm and 1,500 mm annually, heavy rainfall in kharif season (May-September) and scanty in rabi season (October-March). Severe storms are unusual but tornadoes have struck the southern areas sometimes. During the month of December, January and February there was no rainfall. During the period of study the average maximum temperature was 32^0 C and average minimum temperature was 20^0 C. Details of the meteorological data in respect of temperature, rainfall and relative humidity during the period of experiment were collected from Bangladesh Meteorological Department, Agargaon, Dhaka (Appendices-III).

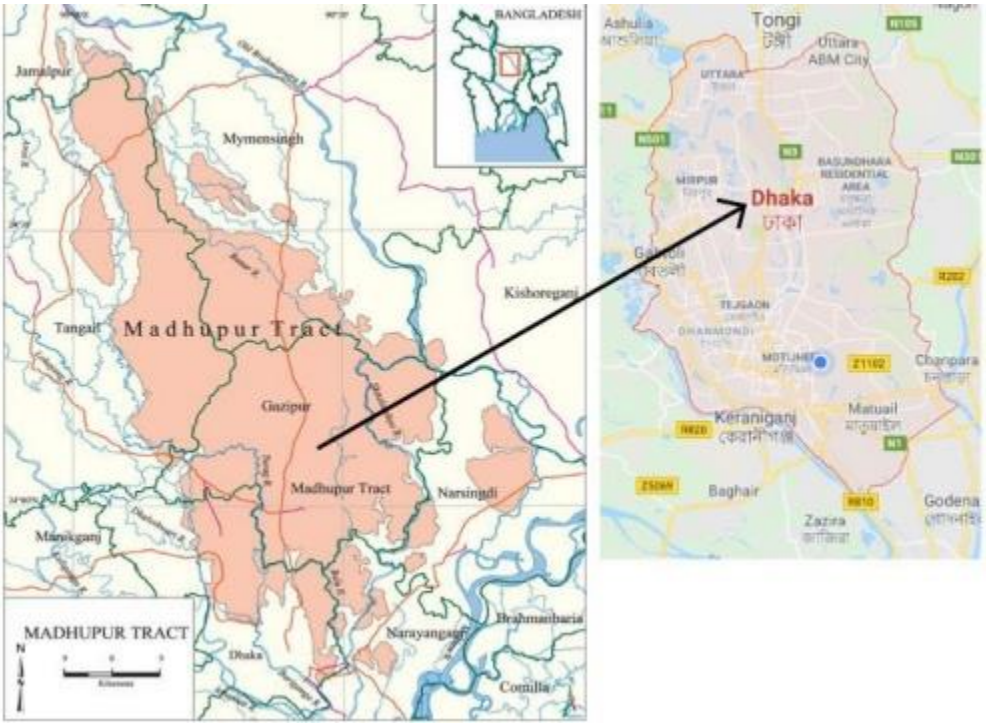


Figure 1. Madhupur tract, AEZ No-28

3.4. Sampling

Blast disease became more devastating in most of the rice growing areas of Bangladesh since last few years. Among them northern part of Bangladesh was mostly affected. Samples were collected from three blast reported upazilla/sub-station under the selected region of greater Dinajpur including Dinajpur, Thakurgaon and Panchgarh. In this case diseased plant parts were collected like leaves, node, panicle and grain and samples were tagged with necessary informations so that it could be helpful for further study. In this study two experiments were conducted:

1. Lab experiment
2. Pot experiment

3.5. Lab experiment

3.5.1. Design of the experiment

The diseased samples were placed in blotter paper method and in artificial culture media under the control conditions with complete randomized design (CRD).

3.5.2. Equipments used

The following equipments and materials were used in present investigation:

1. Incubator for incubation

2. Autoclave for media sterilization

3. Hot air oven for glassware sterilization

5. Inoculation needle, forceps, needles, blades, threads, scale, aluminium foil

6. Laminar air flow for isolation and purification

7. pH meter

8. Electronic weighing balance

9. Refrigerator

10. Spirit lamp

3.5.3. Cleaning and sterilization of glassware

For all the laboratory studies, Pyrex glassware was used. The glassware were first cleaned with a detergent, followed by thorough cleaning with tap water. The cleaned glass wares were placed in potassium dichromate solution for 24 hours and finally rinsed with distilled water for 3-4 times. Then they were air dried and sterilized in hot air oven at 160-170ºC for one hour. Media used in the study were sterilized in an autoclave at 121ºC temperature under15 psi for 30 minutes. Work benches were sterilized by using 70% ethanol. Other tools such cork borer, scalpel, Forceps and needles were sterilized by flame.

3.5.4. Comparative Study of Different Culture Media

In total three culture media were used in this experiment. These three media and their preparation process are given below:

3.5.4.1. Preparation of Potato Dextrose Agar (PDA) Media

PDA media was prepared according to standard protocol as describe by Ricker and Ricker, 1936. The composition of PDA media is given below:

Ingredient	Quantities
Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled Water	Upto1 litre

200 g disease free and fresh potato was cleaned and washed thoroughly under running tap water and then rinsed with sterilized water. Cleaned potatoes were taken in sterilized vessels and peeled with sterilized and sharp knife. Peeled potato

were sliced and then boiled in 500 ml distilled water for half an hour. It was later filtered using a markin cloth. Dextrose was added and the extract was made upto one liter. 20g Agar powder was weighted and then added gently, stirred well using a glass rod to get uniform distribution. The media thus obtained was dispensed equally into 250 ml. @ 100 ml conical flask and sterilized. Then 1 ml lactophenol was added after autoclaving in laminar airflow cabinet.

3.5.4.2. Preparation of Carrot Rice straw Dextrose Agar (CRDA) Media

CRDA media is a noble media for the mycelial growth and sporulation of *Pyricularia oryzae* .Composition is given below:

Ingredient	Quantities
Carrot infusion	200 g
Rice straw	50 g
Dextrose	20 g
Agar	20 g
Distilled Water	1 litre

200 gm disease free fresh carrot was cleaned and washed thoroughly under running tap water and then rinsed with sterilized water. Cleaned carrots were taken in sterilized vessels and peeled with sterilized and sharp knife. Peeled carrots were sliced and boiled in 500ml distilled water for half an hour. It was later filtered using a markin cloth. Then 50 gm of rice straw was cut into small pieces with a sharp sterilized knife and boiled in 150ml distilled water for 20 minutes. It was later filtered using markin cloth. Then carrot extracts and rice straw extract mixed together by shaking thoroughly. Again filtered with markin cloth. Dextrose was added and the extract was made up to 1 liter. 20 g Agar powder was weighted precisely and then added gently, stirred well using a glass rod to get uniform distribution. The media thus obtained was dispensed equally into 250 ml. @ 100 ml conical flask and sterilized.

3.5.4.3. Preparation of Corn Meal Agar media

Corn meal agar media was prepared according to standard protocol as describe by Pollack and Benham, 1960. The composition of Corn meal agar media is given below:

Ingredient	Quantities
Corn Meal Agar	17 gm
Distilled Water	1 litre

17 gm of corn meal agar was suspended in 1 liter distilled water. Heated and boiled to dissolve the medium completely. Sterilized by autoclaving at 15 Ibs pressure (121^0C) for 15 minutes. Then cooled to 45-50^0 C. Mixed well and poured into sterile petri plates.

3.5.5. Isolation of *Pyricularia oryzae*

Fungi was isolated by tissue planting method (Agrios, 2006). Diseased rice leaves and neck portions of the infected stems which were collected from the field, were cut into small pieces 5mm sized along with healthy portion. Cut pieces were sterilized by the surface disinfectant Sodium hypochlorite (1%) for 3 minutes (Ellis, 2001). After sterilization the cut pieces were washed three times with sterilized water. The cut pieces were placed on sterile paper towels to remove excess water. The cut pieces were then placed on the blotting paper under sterile condition. The plates were labeled with necessary information and placed in the incubation chamber for 7 days at 25 $\pm1^0$C. During incubation they were exposed to NUV light for 12 hours per day. After 7 days of incubation, the fungi was grown on culture media. A portion of culture was taken on slide and observed under compound microscope and identified the pathogenic fungi that was *Pyricularia oryzae* with the help of pertinent literature (Mew and Gonzales, 2002; Ellis 2001; Barnet and Hunter, 1972).

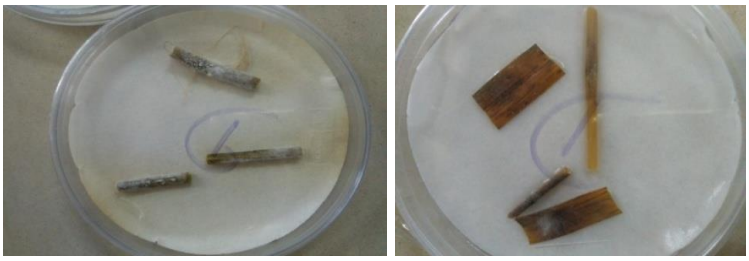


Figure 2. Blast infected Cut pieces of Rice leaf samples on blotter paper

The growing mycelia on petri plates were then placed in PDA media and CRDA media and then were incubated at 25 to 26°C for 7 days. After 7 days the fungi grown on the culture media. A portion of culture was taken on slide and observed under microscope and identified the pathogenic fungi that is *Pyricularia oryzae* with the help of pertinent literature (Mew and Gonzales, 2002). The growing mycelia on potato dextrose agar plates and Carrot rice straw dextrose agar plates were incubated at 25^0 to 26°C for 48 to 72 hours to induce sporulation of the fungus. For cultural characterization the plates were further incubated for fifteen days. A piece of the young sporulated fungus was picked using a needle, under binocular microscope. The sector of growing mycelia was placed on a slide with a drop of glycerine, the mycelia was covered by a cover slip and observed under the microscope for classical characterization. Single spores were carefully picked and transferred to PDA and CRDA in Petri dishes for incubation under 25^0 to 26°C for a maximum of 48 hours. Replicates of the same were stored as spores asceptically on slides and kept under sealed polythene bags. Then from the pure culture a small portion of mycelia placed on corn meal agar media and kept in incubation chamber under 25^0 to 26^0 C for a maximum of 72 hours. The whole process was gone through sterile condition. After 3 days of pathogen placement no mycelial growth was observed in Corn Meal Agar media. In the next step a small portion of mycelia from the pure culture was collected by a needle and placed it on CRDA media and then incubated for 3 days under 25^0 to 26^0 C. The best growth was observed in this media. CRDA media is the noble and best media for the culture of blast pathogen (Figure 3).

PDA media **CRDA media** **Corn meal agar media**

Figure 3. Mycelial growth of *Pyricularia oryzae* in selected media

3.5.6. Purification of the pathogen

The mycelia which was grown marginally and developed subsequently was picked up aseptically for sub-culturing. The sub culturing was done at an interval 21 days and preserved at low temperature (5 ± 1^0C) in refrigerator .

3.5.7. Cultural and morphological study of *Pyricularia oryzae*

Isolates were identified based on their morphological growth pattern and spore shape on PDA medium and CRDA media. Morphological variation among the isolates were examined and characterized according to their conidial structure (size, shape, color and septation of conidia) under stereo microscope. Redial growth of mycelia was also studied.

3.5.8. Sporulation of *Pyricularia oryzae*

The fungus was started to sporulate after 25 days of inoculation on PDA and CRDA media. On CRDA media fungus produces satisfactory amount of spores as it is suitable for its sporulation. For counting spores petri plates of CRDA containing pure culture was taken and poured 10 ml water on it and stirred well with a brush. Then the suspension was filtered with a markin cloth and harvested the conidial suspension in a beaker. Again added 10 ml more water and repeated

the same. The mass of spore sedimentation was collected re-suspended with sterilized distilled water and spore density was adjusted to a concentration of 1×10^5spore/ ml using a haemocytometer. A loopful of spore suspension was then placed on a clean slide and a cover slip was placed on it. The rate of sporulation was recorded in three different microscopic fields. Mycelial growth, conidiophore and conidia was observed clearly after staining with lactophenol cotton blue under light microscope at first low power and then with high power objective lens. Microphotographs were taken to show the typical spore morphology of *Pyricularia oryzae* isolates.

3.5.9. Storage of pure culture

Pure cultures were stored at to 4°C temperature in refrigerator for future research work.

3.6. Pot Experiment

The pot experiment was carried out in net house of Plant Pathology Department, SAU, Dhaka-1207. For this study, BRRI dhan 28 was used which was highly blast susceptible variety of rice to conduct the experiment in Rabi season, 2017. The area of the nursery was 50 square meter. Healthy and disease free seeds were collected and sown in nursery bed after treating with Tricyclazole 75 WP @ 1.5 g/ kg of seeds dated December, 2017. Then 25 days old seedlings were transplanted in experimental pot. Each pot contains 3 plants. Seedlings were treated with potassium silicate. Total number of treatments were six. Potassium silicate was sprayed in different doses like 0, 1, 2, 4, 8, or 10 g L^{-1} with three replication each number of sprayings. Potassium silicate was collected from respective country dealer. Proper fertilization was done for better growth of plants. The pathogen was inoculated at 35 DAT under proper safety by maintaining inoculation principles and spore density was adjusted to a concentration of 1×10^5 spore/ ml. First reading was taken before first spray and then at 15 days interval for 3 times. Completely Randomized Design (CRD) was used to carried out the experiment.

3.6.1. Seed collection and seedling preparation

Disease free rice seeds were collected from BADC. Variety BRRI dhan 28 was selected for this study because it had been showed high susceptibility to Blast disease caused by *Pyricularia oryzae* (Figure 4). For growing of seedling seed bed was prepared in SAU Agronomy field. Then wet sprouted rice seeds were broadcasted on the prepared seed bed. After proper care of 7 days seeds were germinated. Proper mature healthy seedlings were grown at the age of 30 days (Figure 8).

A **B** **C**

Figure 4. Healthy disease free rice seeds (BRRI dhan 28) and sprouted rice seeds (A. Healthy rice seed**, B.** Sprouting stage **C.** Sprouted seeds)

Figure 5. Prepared seed bed and disease free seedlings raised in seed bed.

3.6.2. Pot preparation and seedling transplantation

For pot experiment 18 pots were collected from nearby market and kept in net house of Plant Pathology Department, Sher-e-Bangla Agricultural University. Pots were then filled with sterile soil. Then different doses of fertilizers were applied to the pot soil like Cowdung 5 kg (250 g /pot), Urea 1kg (50 g /pot),TSP 1 kg (50 g /pot), Gypsum 1 kg (50 g /pot),Boron 100 g (3.3 g /pot), Zinc Sulphate 100 g (3.3 g / pot), then mixed them with soil properly. Then sufficient amount of water was added to each pot in every morning so that the soil became soft and muddy to grow rice seedlings. Thus the pots were prepared.25 days old rice seedlings were transplanted in the ready pots. Each pot contained at least three transplanted rice seedlings (Figure 6).

Figure 6. Prepared pots and transplantation of rice seedlings in pot

3.6.3. Intercultural operations

Different types of intercultural operations were done according to agricultural practices after transplantation to mature stage. These are given below:

3.6.3.1. Gap filling

After one week of transplanting Gap filling was done. The seedlings were taken from the same source and a minor gap filling was done where it was necessary.

3.6.3.2. Weeding

Hand weeding was done for three times. First one was done at 20 DAT (Days after Transplantation) and second and third weeding were done at 40 and 60 DAT respectively.

3.6.3.3. Manure and Fertilizer management

All the fertilizers were applied in basal dose except urea. Urea was applied three times from the beginning of transplantation to the end of maturity.

3.6.3.4. Irrigation water and drainage

Irrigation was done according to necessity. The pots were irrigated by a watering can.

3.6.4. Preparation and spraying of Potassium Silicate (K_2SiO_3)

Potassium silicate was collected from respective country dealer. The preparation process of potassium silicate was carried out in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. At first exact amount of K_2SiO_3 was weighed by a balance and then weighed K_2SiO_3 was added in one litre distilled water for each dose (0, 1, 2, 4, 8, or 10 g L^{-1}). Then it was stirred well by a glass rod. Thus the solution became ready to use (Figure 7 and 8).

In this study in total 6 treatments were selected with 3 replication and the experiment was carried out with CRD design. Potassium silicate was sprayed in different doses like 0, 1, 2, 4, 8, or 10 g L^{-1} with three replication each number of sprayings at two solution pH. First spray was done at 35 days after emergence. Then second spray was carried out before panicle initiation stage and third one before heading stage in the same dose. But greatest reduction on blast incidence was observed at 4 g Si L^{-1}, regardless of solution pH.

Figure 7. Preparation of Potassium silicate solution

Figure 8. Spraying of Potassium Silicate on Rice seedlings

3.6.5. Inoculation of Pathogen

Each pot was inoculated with the test pathogen isolate, Spore suspensions of 1x10 ^{5} spore/ml sprayed on leaves by using hand sprayer (Han *et al.,* 2003). Seedlings sprayed with each isolate were covered with a polythene bag, cheapest and easy process of inoculation. Inoculated plants were kept for incubation in moist chamber at 28 ^{0} C with >95% RH for 72 hours under darkness for penetration of conidia and disease development. The pathogen was inoculated on the 38th DAT under proper safety by maintaining inoculation principles. The whole work was done under controlled condition. Disease incidence was evaluated ten days after inoculation (Figure 9).

Figure 9. Inoculation of Pathogen

3.6.6. Assessment of Disease Incidence of Rice Blast

The disease incidence was recorded during post flowering stage. Data were recorded visually by observing the symptoms. Two plants were randomly selected from each pot and the following parameters were considered for data collection namely; number of tillers /pot and number of diseased tillers /pot.

Disease incidence was calculated by the following formula (Webster and Greer,1992):

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased tillers}}{\text{Total number of inspected tillers}} \times 100$$

3.6.7. Assessment of Disease Severity of Rice Blast

The severity of the disease was examined visually on the whole plants within the quadrants and recorded as the percentage of plant parts (tissue) affected (percentage of blast infection of the plant). Disease severity of Rice blast (*Pyricularia oryzae*) was recorded by SES IRRI, 1996 used a 0-9 scale for predominant lesion type:

0= No lesion observed= Highly resistant =0%
1= 10% leaf area covered by blast spots= Resistant = 10%
2-3=20-30% leaf area covered by blast spots= Moderately resistant = 20-30%
4-5= 40-50% leaf area covered by blast spots=Moderately susceptible= 40-50%
6-7 =60-70% leaf area covered by blast spots= Susceptible= 60-70%
8-9=80-90% leaf area covered by blast spots= Highly susceptible = 80-90%

3.7. Parameter Assessed

3.7.1. Lab Experiment

In lab experiment mycelial growth on different media was observed and radial growth was calculated. The Number of spores were also counted.

3.7.2. Pot Experiment

In pot experiment no. of tiller/ pot and no. of panicle/ pot was counted. Disease incidence and disease severity was also assessed.

3.8. Statistical analysis of data

The data was analyzed by using the “Statistix 10” Software. The mean value was compared according to LSD range test at 5% level of significance. Tables, bar diagram, linear graphs and photographs were used to present the data as and when necessary.

RESULTS

This chapter comprises the explanation and presentation of the results obtained from the lab experiment and the pot experiments on rice blast pathogen *Pyricularia oryzae* and evaluation of the efficacy of the potassium silicate against the blast pathogen.

4.1. Symptomology of different types of rice blast

The samples were collected from selected regions having blast symptoms on leaves, nodes and panicles. The leaves were showed characteristic blast symptoms; brown colored spindle shaped spots with greyish center was found on leaves. In severely affected leaves spots were gradually coalesce together and blighted the whole leaf area as clear in Figure 10 (A). Infection to the node produced purplish lesions called node blast, followed by lesion elongation to both sides of the neck node as clear in Figure 10 (B). Dark brown to black colored lesion was found on infected panicle represented the characteristic panicle blast symptom as clear in Figure 10 (C).

A. Leaf blast **B. Node blast** **C. Panicle blast**

Figure 10. Different types of rice blast symptoms

4.2. Results of lab experiment

4.2.1. Isolation of the *Pyricularia oryzae* from collected samples

Rice Blast disease has been identified as a devastating disease in all over Bangladesh since last few years, greater incidence identified in northern part of our country. Prevalence of rice blast observed in greater Dinajpur district. The study revealed that the samples were collected from the villages which were infected with higher disease incidence and severity combination, had lower yield. At first, Rice blast pathogen, *Pyricularia oryzae* was isolated in Potato Dextrose Agar (PDA) media and purified. At initial stage, the pathogen produced white mycelial growth and later on it turned into black in color as clear in Figure 11. Sporulation rate was very poor in PDA even after 30-35 days of culture maintenance.

Figure 11. Radial mycelial growth of *Pyricularia oryzae* in PDA media at 30 DAI

4.2.2. Mycelial growth of *Pyricularia oryzae* in different selected media

The pathogen was grown in different selected media. The mycelial growth was recorded at 7, 14 and 21days after inoculation. From the study it was found that the highest growth of 60.60 mm, 70.80 mm and 80.14mm was recorded in CRDA media at 7, 14 and 21 days after inoculation, respectively (Table 1 and Figure 13). The second highest growth was recorded in Potato dextrose agar (PDA) medium 60.16 mm at 21 days after inoculation (Table 1 and Figure 12). There was no mycelial growth found in corn meal agar media as clear as in Figure14. So, CRDA and PDA media were found to be the suitable media for mycelial growth of *Pyricularia oryzae*. The best media found for better mycelial growth of

Pyricularia oryzae was in CRDA media. The highest growth was recorded in CRDA media 80.14 mm where 60.16 mm mycelial growth was recorded in PDA media. No growth was observed in Corn meal agar media (Figure 15). From the study, it was revealed that the highest sporulation was found in CRDA media and very poor no. of spores were found in PDA media. The number of spores were counted by a haemocytometer.

The number of spores found in CRDA media was 40.2×10^6 per 20 ml of spore suspension at 30 DAI.

Table 1. Radial mycelial growths of *Pyriculariaoryzae* in different culture media

Media	**Growth(mm)**		
Days of observation	**7 DAI**	**14 DAI**	**21 DAI**
PDA	I-1= 29.8 c I-2=32.13 b I-3= 30.42 c	I-1= 47.6 b I-2= 50.19 a I-3= 50.77 a	I-1= 49.3 b I-2= 50.81 a I-3= 60.16 a
CRDA	I-1= 30.8 c I-2=33.83 a I-3=30.83 c	I-1=57.3 a I-2=52.27 b I-3=52.67 b	I-1=70.13 a I-2=66.00 a I-3=80.14 a
Corn meal agar	0	0	0
CV (%)	2.71	1.69	2.22

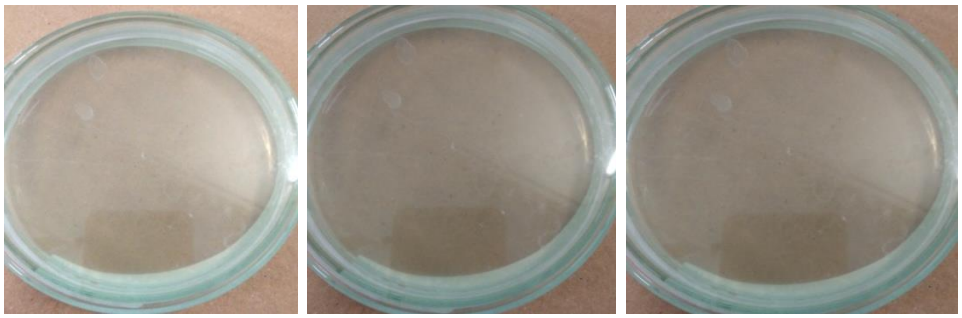
(I-1= Isolate 1 from Dinajpur, I-2= Isolate 2 from Rangpur, I-3= Isolate 3 from Thakurgaon)

7 DAI **14 DAI** **21 DAI**

Figure 12. Radial mycelial growth of *Pyricularia oryzae* in PDA media at 7 DAI, 14 DAI and21 DAI

7 DAI **14 DAI** **21 DAI**

Figure 13. Radial mycelial growth of *Pyricularia oryzae* in CRDA media a 7 DAI, 14 DAI and21 DAI



7DAI **14 DAI** **21 DAI**

Figure 14. No Radial mycelial growth of *Pyricularia oryzae* in Corn meal agar media 7 DAI, 14 DAI and21 DAI

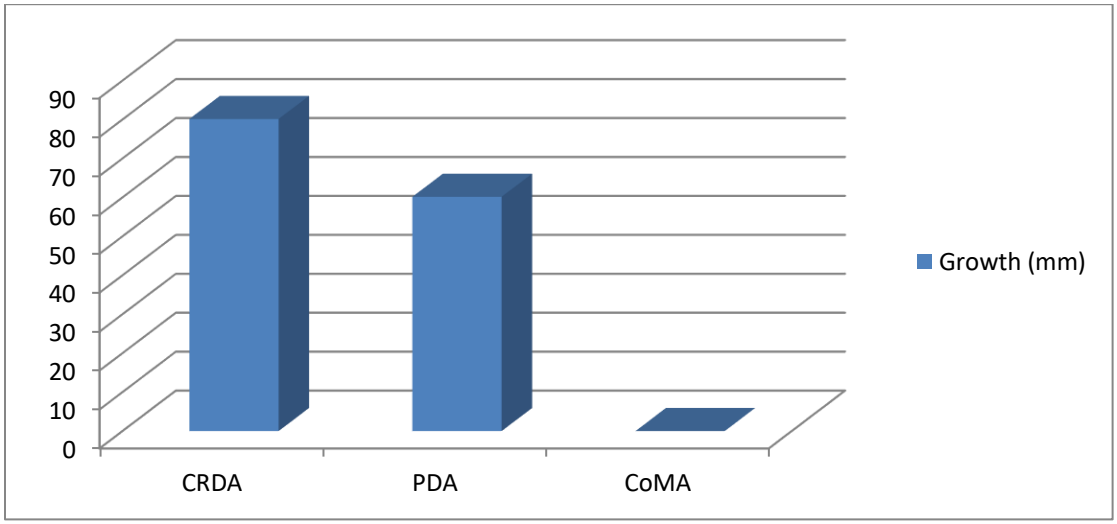


Figure 15. Effect of different media on mycelial growth of blast pathogen of rice

4.2.3. Study of morphological characteristics of *Pyricularia oryzae*

Slide culture and microscopic study was done by using the pure culture of *Pyricularia oryzae* from CRDA and PDA media. Mycelia, conidiophores and conidia were found under the light microscope. The length and breadth of the conidia was measured. The size of conidia was measured about 17.96 - 26.64 µm × 7.36 - 9.22 µm (average 22.42 × 8.59 µm) and 12.06 - 19.95 µm × 5.38 - 9.06 µm (average 16.45 × 7.46 µm) in CRDA and PDA media, respectively (Figure 16). The spore measurements were 15 – 22 µm × 4 – 7 µm (Average, 17.4 µm × 5.2 µm). Mostly 3 celled conidia were found from both CRDA media and conidia were found in infected leaf sample.

Table 2. Number of spore counting in CRDA media at different observations

Observation	Total no. of spore counted
1st @ 30 DAI	40.2×10^5 / 20 ml spore suspension
2nd @ 45 DAI	52.6×10^5 /20 ml spore suspension
3rd @ 60 DAI	75.2×10^5 /20 ml spore suspension

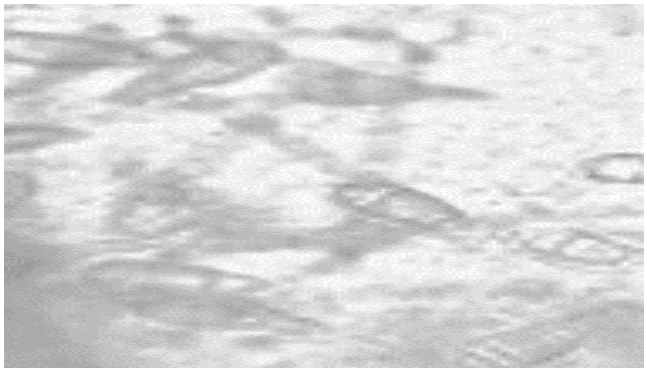


Figure 16. Microscopic view of mycelia, conidiophores, and conidia of Pyricularia on slide preparation from CRDA

Figure 17. Microscopic view of mycelia, conidiophores and conidia of *Pyricularia* on slide preparation from PDA media

4.3. Pot experiment

4.3.1. Effect of Potassium Silicate (K_2SiO_3) on Blast disease incidence of rice under pot conditions

K_2SiO_3 was sprayed as a foliar spray with different doses that described in methodology section. The disease incidence was recorded at 45, 60 and 75 DAS of K_2SiO_3 spraying and at panicle initiation stage. It was revealed that blast disease incidence significantly decreases with the increased of K_2SiO_3 doses. The highest disease reduction was found at 4 g $L^{-1}K_2SiO_3$ spraying and disease incidence was ranges from 1.15-5.5%. It was also observed that K_2SiO_3 doses increased up to 10 g L^{-1} and after 4 g L^{-1} K_2SiO_3 dose disease incidence was slightly increase. The highest disease incidence was recorded in control treatment (55.77 - 90%). The results are presented in Table 3 and Figure 19.

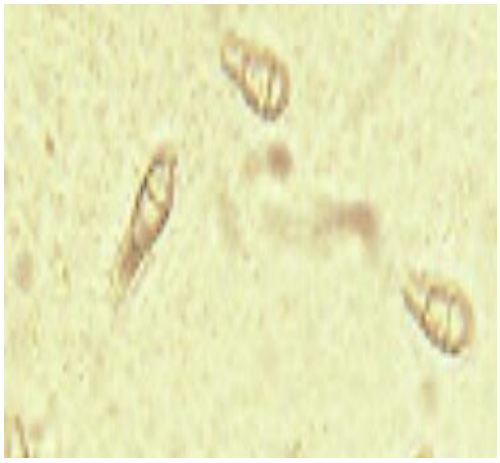
It was also observed that disease incidence results showed the same trend at 75 DAS (Figure 20) and at panicle initiation stage (Figure 21). The highest disease incidence was recorded in control treatment and the lowest was in at 4 g L^{-1} K_2SiO_3 spraying.

Table 3. Effect of Potassium silicate on *Pyricularia oryzae* inoculated rice showing disease incidence (%) under pot condition

Sl. No.	Treatment (K_2SiO_3)	Disease incidence (%) @ 45 DAS	Disease incidence (%) @ 60 DAS	Disease incidence (%) @ 75 DAS	Disease incidence (%) at panicle initiation stage
1	T_1	55.77 a	55.77 a	63.39 a	90 a
2	T_2	2.47 b	3.90 b	6.75 b	40 b
3	T_3	6.52 b	7.76 b	7.75 b	40 b
4	T_4	1.15 b	3.45 b	3.56 b	5.5 f
5	T_5	5.71 b	8.01 b	12.06 b	10 d
6	T_6	3.17 b	6.27 b	10.07 b	20 c
	LSD (0.05)	15.21	15.530	13.58	17.38
	CV (%)	149.46	140.5	103.26	141.03

(T_1= 0 g/L, T_2= 1 g/L, T_3= 2 g/L, T_4= 4 g/L, T_5= 8 g/L, T_6= 10 g/L K_2SiO_3)

Sample collected from control pot and isolated in CRDA media



Pathogenic structure of *Pyricularia oryzae*

Figure 18. Mycelial growth and pathogenic structure of *Pyricularia oryzae* in CRDA media

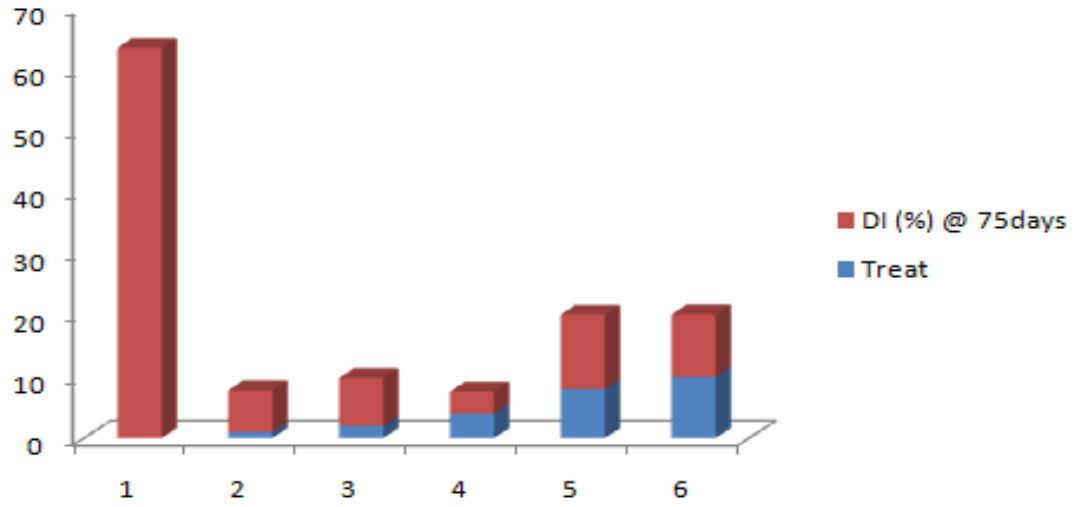


Figure 19. Effect of Potassium Silicate on Blast disease incidence (%) at 75 DAS of rice (BRRI dhan 28) under pot condition

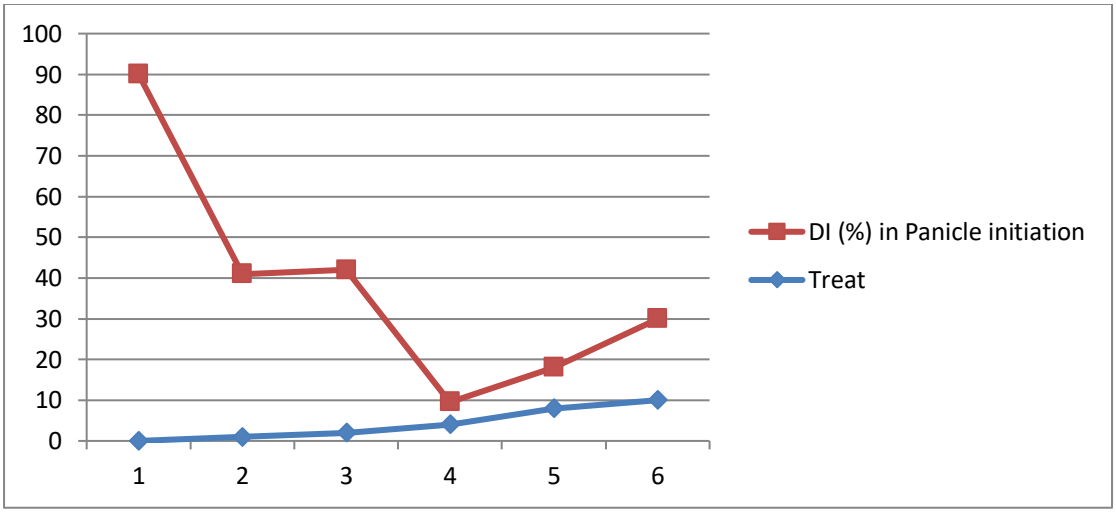


Figure 20. Effect of Potassium Silicate (K_2SiO_3) on disease incidence at panicle initial stage

4.3.2. Effect of potassium silicate (K_2SiO_3) on Blast disease Severity of rice under pot condition

K_2SiO_3 was sprayed as a foliar spray with different doses that described in methodology section. The disease severity was recorded at 45, 60 and 75 DAS of K_2SiO_3 spraying and at panicle initiation stage. It was revealed that blast disease severity significantly decreases with the increased of K_2SiO_3 doses. The highest disease severity reduction was found at 4 g L^{-1} K_2SiO_3 spraying and disease severity was ranges from 3.33-6.0%. It was also observed that K_2SiO_3 doses increased up to 10 g L^{-1} and after 4 g L^{-1} K_2SiO_3 dose disease severity was slightly increase. The highest disease severity was recorded in control treatment (33.33-75%). The results are presented in Table 4.

It was also observed that disease severity results showed the same trend at 75 DAS (Figure 21) and at panicle initiation stage (Figure 22). The highest disease severity was recorded in control treatment and the lowest was in at 4 g L^{-1} K_2SiO_3 spraying.

Table 4. Efficacy of potassium silicate (K_2SiO_3) and *Pyricularia oryzae* inoculation on disease severity (%) of rice (BRRI dhan 28) under pot condition

Sl. No.	Treatment (K_2SiO_3)	Disease severity (%) @ 45 DAS	Disease severity (%) @ 60 DAS	Disease severity (%) @ 75 DAS	Disease severity (%) at panicle initiation stage
1	T_1	33.33 a	33.33 a	46.67 a	75 a
2	T_2	6.67 b	8.33 b	13.33 b	62 b
3	T_3	10.0 b	10.0 ab	10.0 b	53 c
4	T_4	3.33 b	3.33 b	5.0 b	6.0 f
5	T_5	10.0 b	15.00 ab	33.33 ab	11 e
6	T_6	10.0 b	13.33 ab	16.67 ab	42 d
	LSD (0.05)	10.0	11.47	14. 37	15.89
	CV (%)	100	101.11	84.47	89.56

(T_1= 0 g/L, T_2= 1 g/L, T_3= 2 g/L, T_4= 4 g/L, T_5= 8 g/L, T_6= 10 g/L K_2SiO_3)

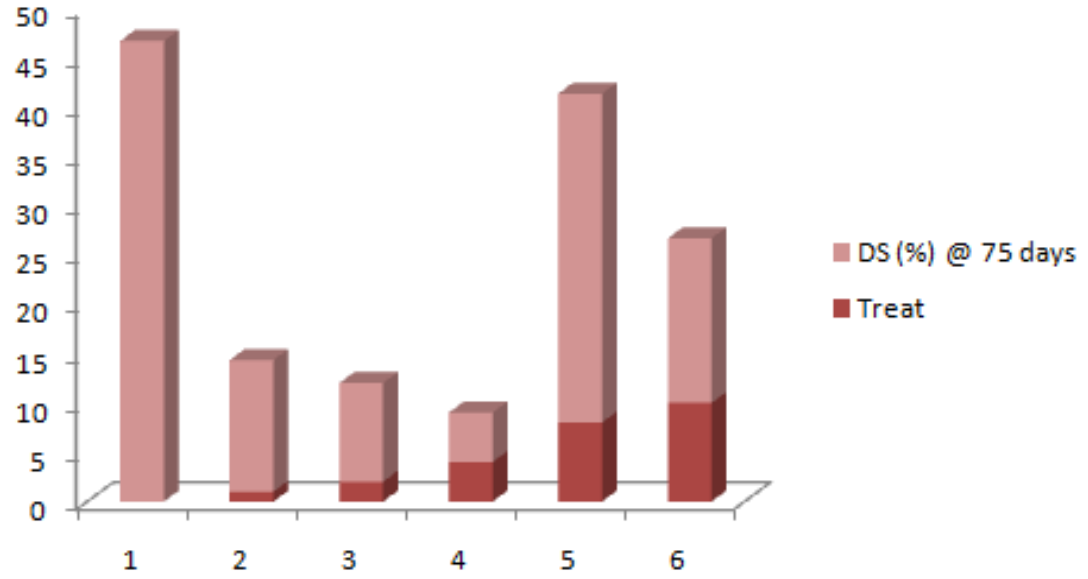


Figure 21. Effect of potassium silicate (K_2SiO_3) on disease severity (%) of rice (BRRI dhan 28) under pot conditions

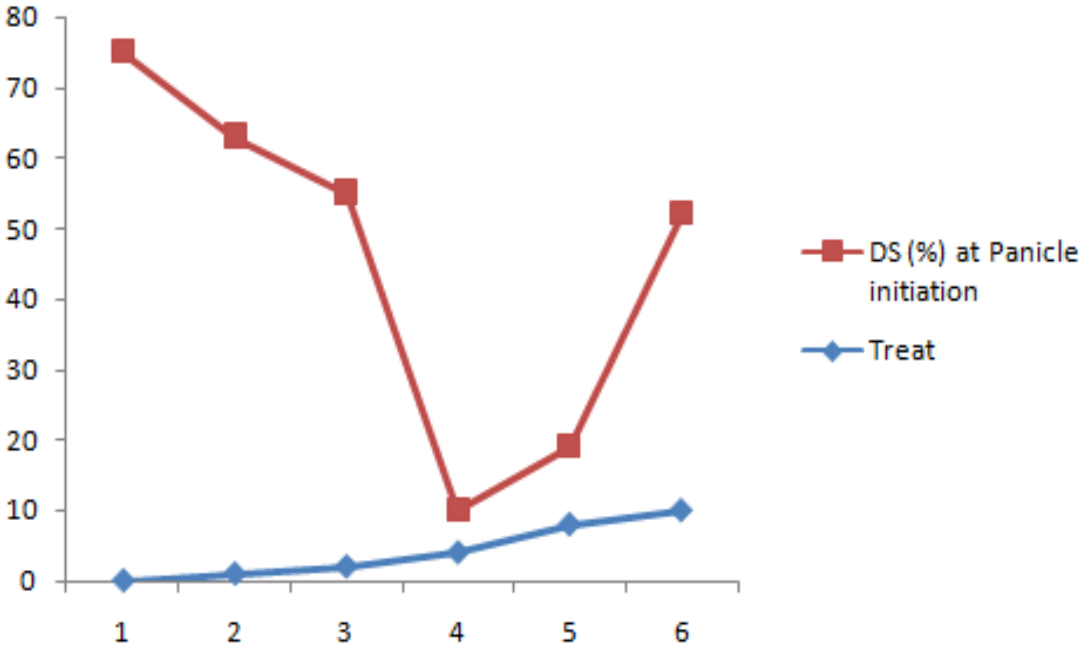


Figure 22. Effect of potassium silicate (K_2SiO_3) on disease severity (%) at panicle initiation stage

4.3.3. Effect of potassium silicate (K_2SiO_3) on panicle initiation and tiller number

A comparison of tiller number/pot and panicle mass/ pot at the vegetative stage and panicle initiation stage indicated that there were significant effects of potassium silicate at any of the doses. In case of tiller number/pot, the results showed a significant variation from 9.67 in the control (without K_2SiO_3 applied) to 30.0 at 4 g $L^{-1}K_2SiO_3$(Table 4, figure 23).

In case of panicle mass/pot, the results showed a significant varied from 8.67 in the control (without K_2SiO_3 applied) to 28.0 at 4 g $L^{-1}K_2SiO_3$ (Table 4, figure 24).

Table 5. Effect of K_2SiO_3 doses applied on the leaves and on number of tiller/pot and panicle mass/pot

Treatment	Number of tiller/pot	Panicle mass/pot
T_1	9.67 c	8.67 c
T_2	23.33 ab	21.33 ab
T_3	25.00 ab	23.33 ab
T_4	30.00 a	28.0 a
T_5	25.66 ab	23.7 ab
T_6	18.33 b	17.33 b
LSD (0.05)	3.78	3.21
CV (%)	83.33	80.24

(T_1= 0 g/L, T_2= 1 g/L, T_3= 2 g/L, T_4= 4 g/L, T_5= 8 g/L, T_6= 10 g/L K_2SiO_3)

Lowest no. of tiller under control **Highest no of tiller @4 g L^{-1} K_2SiO_3**

Figure 23. Rice plants are showing highest tiller number at 50 DAT after spraying Potassium silicate and lowest tiller number under control

Lowest no. of panicle under control **Highest no. of Panicle at 4 g L^{-1} K_2SiO_3**

Figure 24. Plants showing lowest and highest no. of Panicle Initiation before and after Potassium silicate sprayed on rice plants

DISCUSSION

Rice is one of the most important cereal crops of the whole world. It is consumed as a staple food in most of the Asian countries specially in Bangladesh (Yaduraju, 2013). The main constrictions of rice production in Bangladesh is the different kinds of diseases. From the last few years blast of rice caused by *Pyricularia oryzae* broke out in an epidemic form. Its caused great yield losses and that is a great threat for our existance and national economy (Jabeen *et al.,* 2012). There was no resistant variety of rice still identified against blast diserase and no specific treatment to control this disease (Ganesh *et al.,* 2012). To ensure our food security, necessary steps must be taken to manage this disease. To analyse and control blast disease suitable media for its mycelial growth and sporulation must be identified and a suitable chemical and cultural measures need to integrate. Here we studied about mycelial growth of *Pyricularia oryzae* in different selected media and its sporulation and its management with potassium silicate. The whole study was discussed below with different headings and subheadings.

4.4. Sample collection

Prevalence of rice blast observed in greater Dinajpur district. The study was revealed that the samples were collected from the villages which were infected with higher disease incidence and severity had lower yield. Blast infected leaves, nodes and panicles were collected. Rice leaves infected with blast were collected by Bonman *et al.,* (1987) and isolated by placing each lesion in a moist petri dish and incubated at 25^0C until sporulation. Another work done by Correa *et al.,* (1993) collected leaves and panicles infected with rice blast from rice cultivars obtained from germ plasm bank at the Centro Internacional de Agricultura Tropical (CIAT) and the International Rice Research Institute (IRRI).

4.5. Mycelial growth and sporulation of *Pyricularia oryzae* in different selected media

The findings of this study was find out the best culture media for culturing *Pyricularia oryzae*. Three media were used viz. PDA, CRDA and Corn Meal Agar media. The best results were found in CRDA media. In CRDA media mycelia growth and sporulation rate was higher than PDA media. Sporulation was very poor found in PDA media even after 30-35 days of culture maintenance.

PDA media was also used for isolation of *Pyricularia grisea* by (Motlagh and Javadzadeh, 2010), Vanaraj *et al.* (2013) and Suman dutta *et al.* (2017), and they also observed almost same colored mycelial growth in PDA media but no found any sporulation even after 2-3 months of culture maintenance in proper temperature and moist conditions. These results area also in agreement with Ravindramalviya (2014), who studied that PDA and Oat meal agar media supported maximum mycelial growth of *P. oryzae* after 168 hours of incubation.

4.6. Study of morphological characteristics of *Pyricularia oryzae*

The size of the conidia was much higher from leaf sample than in the media. The size of conidia measured about average 22.42 × 8.59 µm and average 16.45 × 7.46 µm from leaf sample and media. Three isolates were found. The mycelium in cultures was first hyaline in color, then changed to olivaceous except isolate no. 3 firstly it was whitish in color then gradually turn into pinkish color.

Mijan Hossain, (2000) also observed that mycelium in cultures was first hyaline in color, then changed to olivaceous, 1– 5.2 µm in width, septate and branched. The spore measurements were 15 – 22 µm× 4 – 7 µm (Average, 17.4 µm × 5.2 µm). Mostly 3 celled conidia were found from both CRDA media and conidia were found in infected leaf sample.

4.7. Effect of potassium silicate (K_2SiO_3) on Blast disease Incidence of rice under pot conditions

The lowest disease incidence was found at 4 g L^{-1} K_2SiO_3 spraying and disease incidence was ranges from 1.15-5.5%. It was also observed that K_2SiO_3 doses increased up to 10 g L^{-1} and after 4 g L^{-1} K_2SiO_3 dose disease incidence was slightly increase. The highest disease incidence was recorded in control treatment (55.77 - 90%).

Similar results were observed by Bonman *et al.* (1987), who stated that the major mechanism for reduction of mildew on grapes was a direct effect of K_2SiO_3 foliar hindering the development of the pathogen, thus affecting its propagation. Research done in organic soils in southern Florida (USA), demonstrated that Si containing fertilization on rice reduced blast incidence 17 to 31% and helmintosporiosis 15 to 32% in relation to a non-fertilized control (Dey *et al.*, 2013). Therefore, Si containing fertilization can reduce or eliminate fungicide spraying in a culture, depending on disease severity. Studies relating powdery mildew reduction with foliar Si fertilization were done on cucumber, melon, and eggplant by Manibhushan *et al.* (1992). According to these authors, leaf spraying with potassium silicate reduced the number of fungus colonies on the leaves. Application of K_2SiO_3 also increased the no. of tiller and panicle per pot. Hossain and Kulakarni (2001) evaluated the efficacy of K_2SiO_3 against *Pyricularia oryzae*, it appeared as the highly effective fungicide that minimize the mycelial growth of the fungus.

4.8. Effect of potassium silicate (K_2SiO_3) on Blast disease Severity of rice under pot conditions

The lowest disease severity was found at 4 g L^{-1} K_2SiO_3 spraying and disease severity was ranges from 3.33-6.0%. It was also observed that K_2SiO_3 doses increased up to 10 g L^{-1} and after 4 g L^{-1} K_2SiO_3 dose disease severity was

slightly increase. The highest disease severity was recorded in control treatment (33.33-75%). The highest disease severity was recorded in control treatment and the lowest was in at 4 g L^{-1} K_2SiO_3 spraying.

K_2SiO_3 increase in plants such as rice, which accumulates in its dry matter. These increases, although not detectable, could have a positive effect on reducing disease severity (Manibhushan *et al.*,1992) also observed the formation of a coating on the leaves after spraying Potassium Silicate, suggesting that the formation of this "film" would strengthen the cuticle activity as a mechanical barrier to pathogen penetration.

4.9. Effect of potassium silicate (K_2SiO_3) on panicle initiation and increase of tiller number

There were significant effects of potassium silicate at any of the doses on tiller number/pot and panicle number/ pot at the vegetative stage and panicle initiation stage. The results showed a significant variation in case of tiller number /pot ranges from 9.67 in the control to 30.0 at 4 g L^{-1} K_2SiO_3. In case of panicle number /pot, the results showed a significant varied from 8.67 in the control to 28.0 at 4 g L^{-1} K_2SiO_3.

These results also commenced to one previous experiment that conducted by Du Xinfa *et al.*, (1995). They found that the general dry matter average on the experiment was 52.46 g per pot, and each pot contained 8 plants, thus 6.56 g. per plant. Considering that a conventional rice crop contains about 45 plants per linear meter, and a row spacing of 17 cm. The pH conditions did not affect leaf Si absorption by rice. The application of Potassium silicate as a foliar spray reduced rice blast incidence.

SUMMARY AND CONCLUSION

The study was comprised to develop reproducible protocol for the mycelial growth and sporulation of *Pyricularia oryzae* to determine the cultural and morphological variability among the isolates of the pathogen. So far, BRRI dhan 28, 32, 33, 44, 45 are the varieties to be tolerant or resistant to blast disease in Bangladesh. According to last two years reports regarding rice blast here, these varieties are no more tolerant or resistant to blast pathogen, *Pyricularia oryzae*. So, currently no reports are available about high yielding commercial varieties or advance rice lines in Bangladesh has shown durable resistance against blast fungus.

In this study a best culture media was identified for the growth of Blast pathogen properly. Three types of media were used such as PDA, CRDA and Corn Meal Agar media. Among these three media best growth and sporulation was observed in CRDA media. Pathogen was isolated again and to determine the cultural and morphological variability for characterization as a new isolates. Three isolates were found. Length and breadth of the conidia of blast pathogen (*Pyricularia oryzae*) was measured from both CRDA media and from lesion developed by inoculation of that culture in the rice leaves. The size of the conidia was much higher from leaf sample than in the media. The size of conidia measured about average 22.42 × 8.59 µm and average 16.45 × 7.46 µm from leaf sample and media, respectively. The spore measurements were average, 17.4 µm × 5.2 µm. Mostly 3 celled conidia were found from both CRDA media and conidia were found in infected leaf sample. Three isolates were found. The mycelium in cultures was first hyaline in color, then changed to olivaceous which 1– 5.2 µm in width, septate and branched. except isolate no. 3. Firstly it was whitish in colour then gradually turn into pinkish color. So from this study, it had been clear that the pathogenic races were changed due to the climate change effect.

It had been reported that the fungicides available in Bangladesh market are not working effectively against the new races of *Pyricularia oryzae*. Beside of this

development of the new rice variety against the new races of *Pyricularia oryzae* was difficult and time consuming for rice breeders to breed for resistance to current races. In this situation new chemical like potassium silicate (K_2SiO_3) at 4 g L^{-1} showed the best option to manage the blast disease of rice. So it was assayed or found out the suitable chemical to control this fungus, which was prime goal of the study. However, from this study two major outputs had been achieved; one was suitable culture media (CRDA media) that can be used for isolation of blast pathogen and another is effective new chemical, Potassium silicate (K_2SiO_3) to manage the blast disease.

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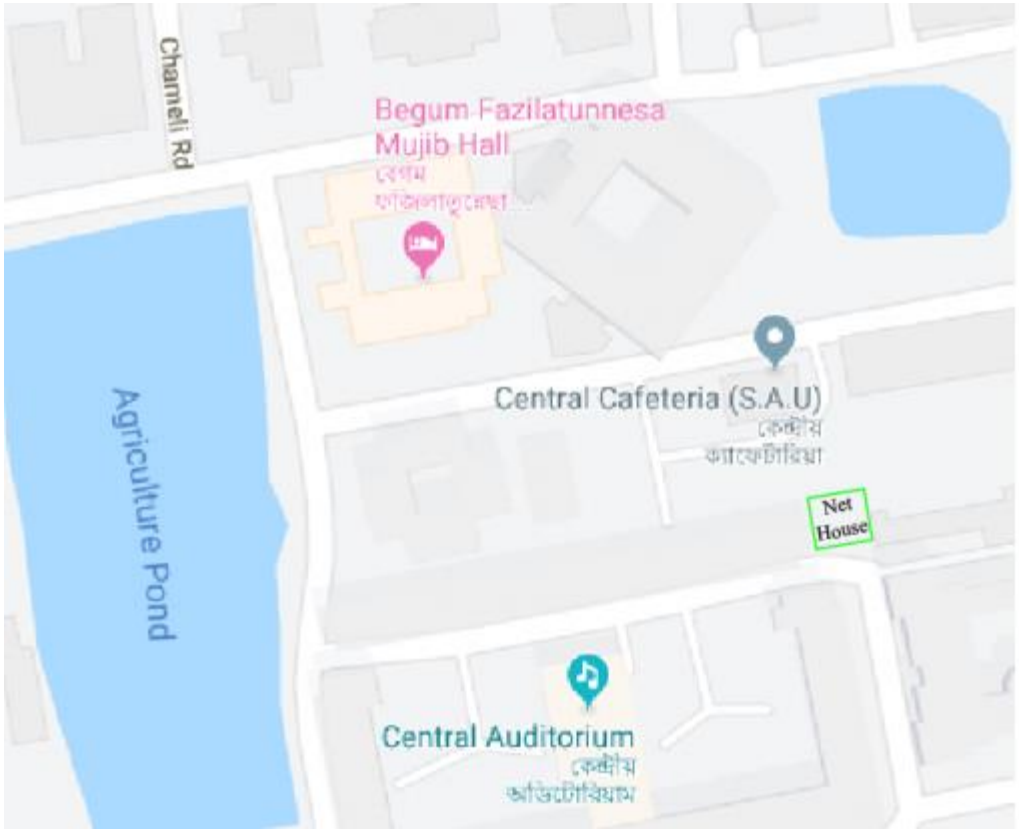
APPENDICES

Appendix-I. Map showing the experimental site under study



Appendix-II. Physiochemical properties of soil, used in the experimental Pots

Characteristics	Value
Sand (%)	25.67
Silt (%)	53.86
Clay (%)	20.48
Texture	Silty loam
pH	5.7-7.1
Organic carbon (%)	0.30
Organic matter (%)	0.55
Total N (%)	0.028
Phosphorus(µg/g soil)	23.59
Exchangeable K (milliequivalents/100 g soil)	0.61
Sulphur (µg/g soil)	28.45
Zinc (µg/g soil)	2.32

Source: Soil Resources Development Institute (SRDI), Dhaka-1207

Appendix-III. Monthly average relative humidity, maximum and minimum temperature, rainfall and sunshine hour of the experimental period (October 2017- March 2018

Month	Average RH (%)	Average Temperature (ºC)		Total Rainfall (mm)	Average Sunshin e hours
		Min.	Max.		
October	79	25	32	175	6
November	65	21	30	35	8
December	74	15	29	15	9
January	68	13	24	7	9
February	57	18	30	25	8
March	57	20	33	65	7

(Source: Bangladesh Meteorological Department (Climate & weather division), Agargaon, Dhaka-1207).

Appendix- IV. Experimental Pots with rice plants in Net house

Appendix-V. Control pot infected with Leaf Blast

Appendix-VI. Healthy pot with no Blast Symptoms after sprayed with 4 gm Potassium Silicate